Original Article

The Human Capsaicin Model of Allodynia and Hyperalgesia: Sources of Variability and Methods for Reduction

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Abstract

Intradermal and topical application of capsaicin have been used to study mechanisms of mechanical allodynia (MA) and pinprick hyperalgesia (PPH) and the efficacy of drugs in relieving these symptoms. However, it is associated with significant inter- and intra-subject variability. In order to improve the model's sensitivity, we examined several potential sources of variability of capsaicin-evoked MA and PPH in healthy volunteers, including skin temperature fluctuations, method (intradermal vs. topical) and site (volar forearm vs. foot dorsum) of administration.

In study I, 12 subjects received, in a 6-session, randomized, crossover trial, 1) 250 μg of intradermal (ID) CAP to the volar forearm with skin temperature fixed at 36°C (36 ID). 2) 250 μg ID CAP with varying skin temperature (VT ID), or 3) 250 μl of 1% CAP patch placed on the skin at 36°C. The resulting MA and PPH areas observed with each method were measured. In study II, a 4-session, randomized crossover trial, 12 subjects were given 100 μg ID CAP in the volar forearm or foot dorsum and subsequent areas of MA and PPH recorded.

In study I, 5/12 subjects had small MA areas ($\leq$ 5 cm^2) and one subject had small PPH areas in at least 4/6 sessions. The most consistent intra-subject responses were seen with the 36 ID method. Correlation coefficients for the two sessions using the same method of administration were: MA; 36 ID $r = 0.83$, VT ID $= 0.19$. Topical $r = 0.81$; PPH: 36 ID $r = 0.93$; VT ID $r = 0.38$, Topical $r = 0.78$. In study II, 4/12 subjects had little MA for both forearm and foot though all subjects developed PPH. However, greater intra-subject consistency (MA: foot: $r = 0.84$; arm: $r = 0.49$; PPH: $r = 0.87$; $r = 0.39$) and significantly larger areas of MA (15.8 ± 4.2 vs 9.1 ± 2.5, $p < 0.05$) were seen with the foot. (PPH: foot: 28.9 ± 6.7; arm: 21.6 ± 4.2, NS).

Large variability exists among subjects receiving CAP, with some developing minimal MA. However, these subjects may be screened out prior to entry, increasing the sensitivity of the model, which may be further improved by clamping the skin temperature. J Pain Symptom Manage 1998;16:10–20. © *U.S. Cancer Pain Relief Committee, 1998.*

Key Words

Capsaicin, mechanical allodynia, pinprick hyperalgesia, experimental pain model

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Introduction

Intradermal injection or high-dose topical application of capsaicin, the purified active ingredient of chili peppers, produces a remarkable set of sensory changes, including ongoing burning and aching pain, thermal hyperalgesia close to the injection site, and mechanical allodynia (MA) and pinprick hyperalgesia (PPH) in the surrounding skin.[1-4] Researchers have used capsaicin in humans to evaluate the mechanisms of MA and PPH[2,3,5,6] and to study the efficacy of drugs in relieving MA and PPH.[7,8]

The sensitivity of capsaicin models for such experiments is somewhat limited by significant variability in the areas of MA and PPH.[7,9] In order to make the most of this tool, methods that eliminate some of the variability would be useful. We therefore set out to compare the variability in sensory changes following several different methods of capsaicin administration. Because several investigators[2,3,9] have shown capsaicin-evoked allodynia or hyperalgesia to be temperature dependent, we evaluated the effects of closely controlling the skin temperature at the application site. Another cause of variation in the response to intradermal injection might be slight variations in depth of injection or leakage of solution from the injection tract. Topical capsaicin application over a larger area might be more resistant than intradermal injection to these variations, and we directly compared topical to intradermal administration to assess this possibility. Sensory changes induced by capsaicin may also vary depending upon the body area to which it is administered. Both the dorsum of the foot[5,6] and the volar forearm[1,2,10] have been used. We compared the size and variability in MA and PPH areas obtained by administering intradermal capsaicin to the foot dorsum and volar forearm.

Methods

Study 1: Comparison of Three Methods of Capsaicin Administration

Twelve normal volunteers completed this six-session, randomized, crossover study comparing three different methods of capsaicin (CAP) administration (two sessions each): (a) 250 μg intradermal (intradermal) CAP to the volar forearm with the skin temperature stabilized at 36°C with a heat lamp (36 ID), (b) 250μg intradermal injection of CAP to the volar forearm with the skin temperature left variable (variable temperature, VT ID), and (c) topical application of a patch with 1% CAP in 70% EtOH (250 μL) for 30 min with skin temperature stabilized at 36°C (topical). Sessions were separated by at least 2 days. All subjects were pain-free and had not used analgesics in the 24 h prior to an appointment.

Subjects were placed in a seated position with the forearm resting on a pillow positioned parallel to the floor at the level of the umbilicus. Skin temperature was monitored throughout all sessions using a thermistor placed 1 cm from the injection/topical site. Skin temperature was fixed using a thermocoupled heat lamp in the 36 ID and topical sessions.

The surface from the antecubital to the wrist crease of both forearms was divided transversely into proximal, middle, and distal thirds. Block randomization was used and each of the six skin areas was used once. Injections were given along the midline of the volar surface, avoiding any veins. Twenty-five μL of CAP (250 μg) (Fluka, Roconocow, New York), reconstituted to 10 mg/mL in Tween-80 using the method of Simone et al.,[1] was injected intradermally using a 27-gauge tuberculin needle after sterile skin preparation with alcohol. Capsaicin was stored as 0.2-mL aliquots in amber-colored vials at −4°C until 10 min prior to administration. The capsaicin was assayed by high performance liquid chromatography for stability at regular intervals. At 9 months, greater than 85% of capsaicin was found to be present. Maximum duration of storage was 1 year.

In the fixed-temperature sessions, the proposed injection or topical site was warmed to and stabilized at 36°C (range, 35.8°–36.2°C) for the entire session beginning 5 min prior to injection. For the topical administrations, we used the method described by Koltzenburg et al.[3] A 2 × 2 cm cellulose adhesive patch was saturated with 250 μL of 1% CAP in 70% ethanol/saline. The 1% solution was mixed immediately prior to each administration using a 10% capsaicin in 100% EtOH stock solution brought to room temperature. The skin was then cleaned with alcohol and the patch placed on the skin. The patch was then covered with an occlusive dressing and left in place for 30 min.

Pain was measured using a 20-cm visual ana-

logue scale (VAS). Pain was measured immediately following intradermal injection and 10 sec prior to patch removal. Pain scores were subsequently obtained every 5 min prior to MA and PPH testing for a total of 90 min. Mechanical allodynia (MA) and pinprick hyperalgesia (PPH) were assessed every 5 min for a total of 90 min following injection or patch removal. MA was assessed using a no. 2 flat, acrylic artist's brush. To ensure beginning in an area of normal sensation, brush sensation of the injected arm approximately 8 cm from the injection or patch site was compared to the brush sensation of the uninjected arm 5 min following injection or patch removal. MA was assessed by dragging the brush lightly at 1 cm/sec from the areas with normal brush sensation toward the CAP administration site in a pattern of eight radial spokes. Subjects were instructed to indicate "when the brush first begins to cause pain or discomfort." These points were marked on the skin with a water-soluble marking pen. PPH was assessed using a 1.5-inch sterile safety pin pressed onto the skin to begin to cause an indentation, starting in an area with normal pinprick sensation, as compared to the opposite arm, approximately 8 cm from the site of CAP administration. Pinpricks were given 1 cm apart, moving toward the site of CAP administration at a rate of one pinprick every 2 sec. Subjects were instructed to indicate "when the pin caused a greater or changed pain sensation compared to the baseline pinprick sensation." If the area was described as a changed sensation, subjects were asked to describe what they felt. Only if they described a pain sensation (for example, burning as well as the pinprick sensation or increased pain with the pin) was this considered the beginning of the PPH area. These points were marked on the skin with a marking pen. Subjects did not observe the sensory testing; eyes were closed or averted from the testing site. At the end of each testing session, the pen marks were traced onto acetate sheets placed over the volar forearm and the points connected to form polygons. These sheets were then photocopied onto standard photocopy paper and the paper areas of MA and PPH were cut out and weighed. The obtained weights were converted to areas by comparing them to the known weight of a known area of the paper.

Study 2: Effects of Location of Intradermal Capsaicin: Forearm versus Foot

Twelve normal volunteers completed this four-session, randomized, crossover study. Subjects were given 100 μg (10 μL) of intradermal CAP to the left or right foot mid-dorsum 4–5 cm distal to the anterior tibia or left or right volar forearm in the lower third of the forearm in the midline after sterile alcohol preparation. Skin temperature was fixed at 36°C (range, 35.8°–36.2°C). Trials were at least 48 hr apart. Subjects were seated with their thighs at 90 degrees to the torso and their lower legs at 135 degrees to the thigh so that minimal pressure was present on the posterior leg to prevent compression and the sensation of the "foot falling asleep." A sitting rather than a supine position was chosen based on the hypothesis that greater sympathetic activity would be present in the feet when the feet are in the dependent position. Pain was measured, as described above, immediately following injection and then every 5 min for a total of 60 min. MA and PPH areas were assessed, using the methods described above, every 5 min for a total of 60 min.

The 100-μg dose of CAP was chosen for the second study based on a previous study in our laboratory[6] and a pilot study with two subjects who received both 50-μg and 100-μg intradermal CAP to the foot dorsum in two separate sessions. They each rated the pain associated with the 100-μg dose as barely tolerable and much greater than with the 50-μg dose. In addition, these subjects had previously received 250-μg CAP to the volar forearm and judged the pain on the foot from the 100-μg dose as comparable.

Subjects were enrolled in both of the above studies after approval by the National Institutes of Health (NIH) Human Investigations Committee and after giving informed consent.

Study 3: Blood Flow versus Position

To test the hypothesis that sympathetic activity to the feet varies with different degrees of dependency, the cutaneous capillary blood flow of the foot dorsum was measured with the lower leg placed at 90, 135, and 180 degrees to the thigh in ten normal volunteers. All subjects had light-colored skin. Skin temperature was stabilized at 36°C throughout the study. A laser Doppler flowmeter (Vasomedics, St. Paul, MN, USA) was placed lightly on the dorsum of the

foot corresponding to the areas used in the study above and capillary blood flow was measured in each of the above leg positions with the patient sitting in a quiet room.

Analysis

Within-subject analyses for the three methods of capsaicin administration and for the foot versus arm sites were done using linear regression coefficients. Correlation coefficients between methods were analyzed using the test of significance of the difference between the correlation values.[11] VAS scores and mean areas of MA and PPH over time for both studies were analyzed using analysis of variance with repeated measures. Positional blood flow effects were analyzed using analysis of variance with repeated measures with Bonferroni corrections.

Results

Study 1: Comparison of Three Methods of Capsaicin Administration

The sample population consisted of four women and eight men aged 21–30 years. Three other subjects did not complete the study due to a delayed local skin reaction to the CAP (a few small papules surrounding the site of injection), four subjects did not complete due to inability to tolerate the pain of CAP injection, and two did not complete due to inability to meet the time requirements.

We defined the minimally adequate area of MA and PPH as greater than 5 cm^2 because the intradermal bleb area is approximately 1 cm^2 and the area of the patch is 4 cm^2. Large variability among subjects in MA and PPH to all forms of CAP administration was observed. Six of the 12 subjects developed at least minimally adequate MA areas, defined as greater than 5 cm^2 for a minimum of 15 min, in all sessions (five subjects) or five sessions (one subject). The remaining six subjects had less than 5 cm^2 MA in at least four/six sessions (Table 1). Ten subjects developed adequate areas of PPH (> 5 cm^2) in response to all forms of CAP administration (Table 2). Ongoing VAS scores (averaged over the initial 15 min) also varied widely (Table 3). Little correlation was seen between ongoing VAS pain scores and MA and PPH areas.

Duration of MA and PPH areas varied widely among the subjects and among the methods as well. Areas of MA decreased over time (Figure 1A) and a number of subjects had small areas after 15 min. As previously reported by Simone et al.,[1] duration of PPH areas was much greater than MA; PPH was often still present at the end of the 90-min sessions (Figure 1B). The mean areas of MA or PPH and pain VAS during the initial 15 min of each session (average of the observations at 5, 10, and 15 min) were used as summary statistics to compare methods.

Larger areas of MA and PPH were found with the 36 ID method than with the variable temperature intradermal and topical methods, but these differences did not reach statistical significance (Table 4). The mean skin tempera-

Table 1
Mechanical Allodynia Areas with Each Method

	36°C ID		Variable temp ID		Topical	
Subject	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	0	0	0	0	0	0
2	7.1	3.6	1.2	6.8	0	0
3	10.4	43.6	33.3	10.4	62.4	12.1
4	24.4	50.3	21.0	26.0	14.9	22.5
5	104.4	111.1	21.0	194.1	100.9	93.1
6	0.8	1.1	7.1	0	0	0
7	8.7	0	6.9	6.9	0	21.8
8	0	0	2.3	0	4.4	3.0
9	55.3	83.4	74.4	17.0	25.9	0
10	29.0	29.2	6.0	17.6	9.1	14.0
11	0	0	0	0	0	0
12	21.4	23.9	16.6	23.8	32.4	17.1

Mean areas (cm^2) at 15 min of each subject's sessions with each method.
36°C ID, intradermal capsaicin with skin temperature fixed at 36°C; Variable temp ID, intradermal capsaicin with uncontrolled skin temperature; Topical, topical capsaicin with skin temperature fixed at 36°C.

Table 2
Pinprick Hyperalgesia Areas with Each Method

	36°C ID		Variable temp ID		Topical	
Subject	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	6.4	0	73.5	0	0	0
2	37.0	23.7	22.6	32.9	28.2	36.6
3	76.9	66.6	68.2	51.1	47.0	84.5
4	37.7	70.6	59.9	63.8	36.5	61.8
5	142.0	164.6	44.0	224.9	136.8	189.0
6	8.7	8.5	0	12.4	0	0
7	25.4	25.5	15.9	25.9	21.8	0
8	33.7	44.9	33.3	55.5	76.5	0
9	104.3	153.3	73.8	87.7	107.2	36.9
10	56.7	72.8	57.8	47.8	44.2	44.1
11	141.3	113.5	107.8	154.9	13.8	19.7
12	47.5	44.3	56.7	62.8	60.4	62.6

The mean areas (cm^2) of the initial 15 min of each subject's sessions are shown.
36°C ID, intradermal capsaicin with skin temperature fixed at 36°C; Variable temp ID, intradermal capsaicin with uncontrolled skin temperature; Topical, topical capsaicin with skin temperature fixed at 36°C.

ture in the variable temperature intradermal method ranged from 32°C to 33.5°C over the course of the session, 2°–4°C lower than the 36°C intradermal and topical methods.

Greater between-session consistency in MA areas was observed for the fixed 36°C intradermal as compared to the variable temperature intradermal method ($r = 0.93$ versus $r = 0.06$, $P < 0.01$) and the topical compared to the variable temperature intradermal ($r = 0.75$ versus $r = 0.06$, $P < 0.05$) methods (Figure 2). No significant difference in consistency was seen in the MA areas of the 36 ID and topical methods. More consistency in PPH areas was observed for the 36 intradermal ($r = 0.91$) trials than for the topical ($r = 0.64$) or variable temperature intradermal trials ($r = 0.38$) ($P < 0.05$ for both) (Figure 2). No significant difference in consistency was seen between the variable temperature intradermal and topical methods.

Using a 20-cm VAS, subjects reported slightly greater mean pain over the initial 15 min (excluding the initial VAS obtained 10 sec following injection) with the variable temperature intradermal (11.8 ± 0.9 cm) than the 36 ID (10.9 ± 1.1 cm) or topical (8.8 ± 1.3 cm) methods. These differences were significant between each intradermal method and the topical method ($P < 0.05$ for each) but not between the two intradermal methods.

Table 3
Ongoing Pain (VAS) with Each Method

	36°C ID		Variable temp ID		Topical	
Subject	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	10.0	16.2	6.0	16.2	3.8	9.0
2	17.6	16	15.2	16	7.2	11.4
3	13.2	13.6	13.8	14.8	13.2	4.8
4	10.0	6.4	13.4	17.4	12.4	14.4
5	8.2	7.8	6.0	9.2	6.0	8.4
6	4.8	4.6	6.6	5.8	3.6	4.2
7	11.2	15.0	9.6	15.6	16.0	16.0
8	11.8	5.8	12.4	15.4	2.0	5.0
9	13.8	17.6	16.2	5.8	12.8	14.6
10	11.8	10.0	8.2	10.8	10.2	8.8
11	17.8	10.0	12.6	18.0	6.2	2.6
12	7.0	17.0	13.2	14.8	11.6	13.0

Average VAS pain score (cm) of the initial 15 minutes of each session
36°C ID, intradermal capsaicin with skin temperature fixed at 36°C; Variable temp ID, intradermal capsaicin with uncontrolled skin temperature; Topical, topical capsaicin with skin temperature fixed at 36°C; VAS, visual analogue scale.

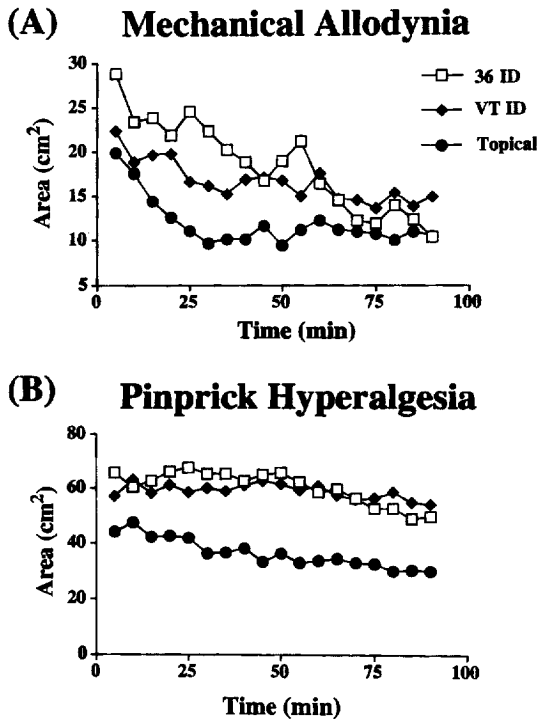

Fig. 1. Comparison of areas of mechanical allodynia (A) and pinprick hyperalgesia (B) with each method of administration over time. Results are shown as the mean of all subjects in cm^2. 36 ID, intradermal capsaicin with skin temperature fixed at 36°C; VT ID, intradermal capsaicin with uncontrolled skin temperature; Topical, topical capsaicin with skin temperature fixed at 36°C.

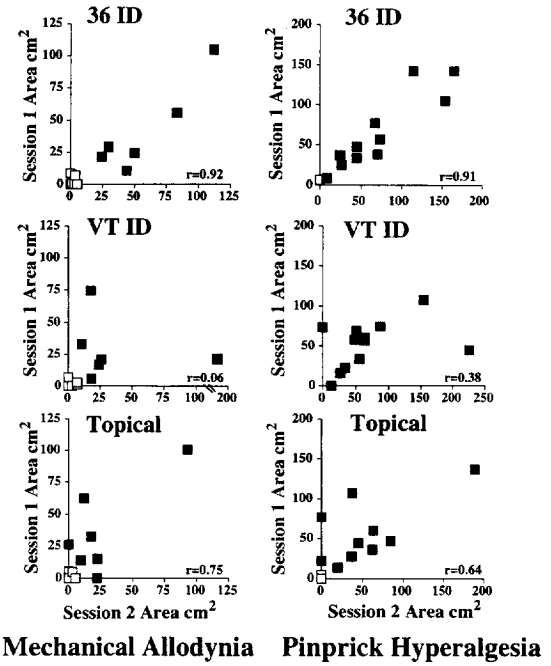

Fig. 2. Between-session comparison of mechanical allodynia (MA) areas (cm^2) (left) and pinprick hyperalgesia (PPH) areas (right) in response to the 36°C intradermal method (36 ID, top), uncontrolled skin temperature intradermal method (VT ID, center), and topical administration method (bottom). The area obtained by each subject in the first trial is plotted against the area of the second trial. Each point represents the mean area during the initial 15 min of each subject's trial. (□) represents subjects who do not meet the minimum criteria greater than 5 cm^2. (■) represents subjects with areas greater than 5 cm^2. Correlation coefficients reflect comparisons only for subjects who have met the minimum area criteria during at least one session.

Study 2: Effects of Location of Intradermal Capsaicin: Forearm versus Foot

The sample population consisted of two women and ten men, aged 21–39 years. All subjects completed the study. With one exception, subjects in this portion of the study were different than those in the preceding study. Because the 36 ID method was found to have the best consistency of the three methods examined in Study 1, this method was used in Study 2.

CAP-evoked MA and PPH areas varied with time (Figures 3A and B). A substantial proportion of the subjects were again noted to have

Table 4
Mean (VAS) Pain Ratings

Method of administration	MA area ± SEM (cm^2)	PPH area ± SEM (cm^2)	VAS ± SEM (cm)
36°C intradermal	25.3 ± 9.6	63.0 ± 14.3	10.9 ± 1.1*
Variable temperature intradermal	20.4 ± 9.0	59.6 ± 11.5	11.8 ± 0.9*
Topical	17.2 ± 8.1	46.9 ± 13.1	8.8 ± 1.3

Mean ± standard error of the mean (SEM) of all sessions for each method of administration for all 12 subjects of mechanical allodynia and pinprick hyperalgesia areas and mean ongoing pain score on a 20-cm VAS over the first 15 min of the sessions.
*$P < 0.05$ as compared to topical.

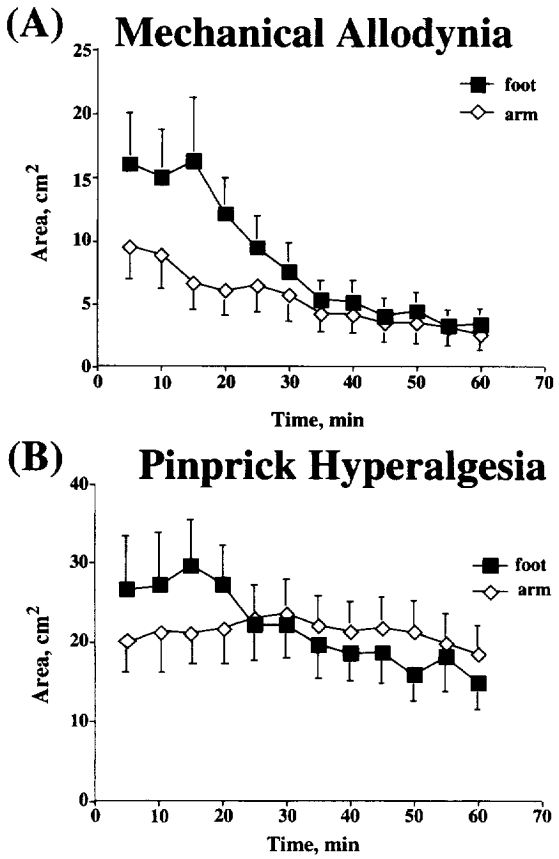

Fig. 3. Comparison of mean areas of mechanical allodynia (A) and pinprick hyperalgesia (B) over time at each site of administration. Results are shown as mean area in cm^2 ± standard error of the mean (SEM).

little or no MA although almost all had PPH. Five of the 12 subjects had MA greater than 5 cm^2 in at least three of the four sessions (Table 5). Eight subjects had PPH 5 cm^2 or more in at least three of the four sessions (Table 6). Between-session consistency for MA was greater in the foot dorsum ($r = 0.84$) than in the arm ($r = 0.49$; $P < 0.05$). As with MA, between-session PPH areas were more consistent in the foot compared to the forearm (foot, $r = 0.87$ versus arm, $r = 0.39$; $P < 0.05$). Larger areas of MA (mean ± SEM: foot, 16.9 ± 4.6 cm^2; arm, 9.1 ± 2.6 cm^2; $P < 0.05$) and PPH (foot, 31.0 ± 7.3 cm^2; arm, 22.4 ± 4.5 cm^2; NS) were found in the foot during the initial 15 min following injection. Mean VAS ratings over the initial 15 min were the same for both locations (11.6 ± 1.1 cm).

In Studies I and II, many subjects had minimal MA and small areas of PPH. Some of the subjects observed that in certain tested areas, the pinprick sensation was actually perceived as decreased compared to the baseline sensation. Three subjects with minimal MA were further tested to delineate this sensory change. As the 36 ID method and the foot dorsum seemed to give the most consistent between-session responses, these subjects were tested using the foot dorsum with skin temperature fixed at 36°C. Sensory testing was conducted as described above except that a calibrated 125.9 g von Frey hair was used in place of the safety pin for punctate sensation. A circular grid with eight equally spaced 8-cm long rays, with punctate stimulation locations separated by 1 cm along each ray, outlining the exact brush and punctate tracts to be tested was drawn on the

Table 5
Mechanical Allodynia Areas: Foot versus Forearm

	Foot dorsum		Volar forearm	
Subject	Trial 1	Trial 2	Trial 1	Trial 2
1	49.0	34.5	14.7	12.3
2	44.8	58.7	34.2	13.3
3	47.3	19.9	18.5	19.9
4	13.0	4.4	0.5	1.2
5	2.6	3.5	1.7	4.3
6	2.1	0	0	0.4
7	0	0	0	0
8	14.6	16.5	23.8	10.4
9	29.5	13.1	8.9	37.0
10	0	14.0	4.2	13.0
11	4.5	0.6	1.2	1.2
12	6.6	0	0	0

Mean areas ± SEM (cm^2) during the initial 15 min of each session at each location.

Table 6
Pinprick Hyperalgesia Areas: Foot versus Forearm

	Foot dorsum		Volar forearm	
Subject	Trial 1	Trial 2	Trial 1	Trial 2
1	45.1	65.4	14.2	21.6
2	51.0	42.1	16.0	54.7
3	123.3	107.9	48.8	51.8
4	12.8	15.4	12.2	0
5	14.3	19.6	15.8	31.1
6	3.1	5.9	14.6	0
7	0.5	4.7	0	0
8	12.7	42.8	31.1	47.2
9	37.9	18.3	17.1	47.4
10	22.7	0	4.6	48.4
11	31.5	7.4	6.2	10.2
12	6.5	4.8	4.6	0

Mean areas (cm^2) during the initial 15 min of each subject's sessions.

subject's foot. Baseline sensory testing was conducted following this grid pattern prior to CAP administration and brush and punctate sensations were perceived as "equal" and "normal" in all tested areas, with no areas of decreased sensation noted. Subjects then received 100-μg intradermal CAP to the foot dorsum. Sensory testing using the brush and von Frey hair was conducted every 5 min for a total of 60 min following the injection. A different foot was tested at each session, with a minimum of 48 h between sessions.

Following each intradermal CAP injection, all three subjects reported intense initial pain. Sensory testing revealed PPH in all three subjects. However, in certain areas the von Frey hair was described as producing a "numbness" sensation. On further questioning, the subjects reported a sensation of dull pressure instead of a pinprick. The location of the decreased sensations was extremely dynamic and often changed from one 5-min testing interval to the next. Areas of von Frey hair-evoked pressure sensation could be adjacent to areas of normal punctate sensations or punctate hyperalgesia. However, in most instances, as the von Frey hair was brought closer to the capsaicin injection site along a test tract, the pressure sensation occurred immediately before the sensation of hyperalgesia, that is, along a tested tract, subjects reported the punctate sensation as "sharp, sharp, pressure, pain." Zones of pressure sensation never fully encompassed the circumference of the site of CAP administration. Little MA was observed. Of note, all subjects reported a feeling of dysesthesia initially with the brush (changed sensation with tingling, judged annoying but not painful) in the tested areas of the foot. Brush sensation reverted quickly to the baseline, non-painful, non-dysesthetic sensation within 10 min. By the end of each of the 60-min sessions, all three tested subjects had normal, baseline sensation to brush and punctate stimuli in the entire area surrounding the CAP injection site.

Because this portion of the study was done in parallel to Studies I and II described above, the remaining subjects enrolled in the two studies (four in Study I, four in Study II) were questioned on all their sensory perceptions to both brush and pin. No subjects reported any decreased perception in the brush sensation. However, six of the eight subjects, both with and without large areas of MA, reported feeling occasional hypalgesia to pinprick testing. Due to the time constraints of the testing intervals, the exact areas of these decreased sensations could not be entirely mapped, but these sensations were again noted to be extremely dynamic.

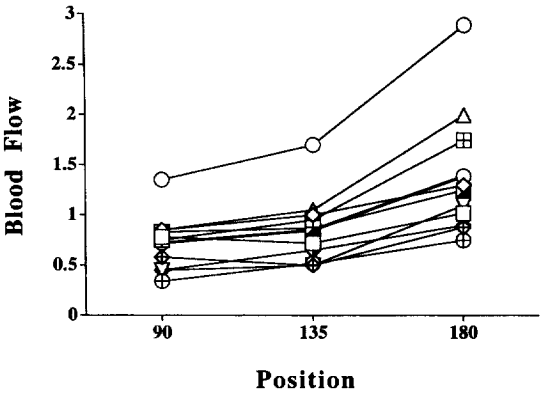

Fig. 4. Blood flow (mL/100 g/min) as measured by a laser Doppler flowmeter on the foot dorsum with the lower leg in various degrees of dependency. Each subject's capillary skin blood flow was measured on the foot dorsum with the lower leg placed at 90, 135, and 180 degrees to the thigh.

Study 3: Blood Flow versus Position

Decreased blood flow was observed with the foot in the two dependent positions (lower leg 90 degrees and 135 degrees to the thigh) as compared to the foot placed on the same level as the thigh, possibly indicating greater sympathetic activity in these positions. Significantly less blood flow was seen between the 90 degrees and 180 degrees (0.72 ± 0.09 versus 1.39 ± 0.21 mL/100 g/min; $P < 0.005$) and 135-degree and 180-degree positions (0.85 ± 0.12 versus 1.39 ± 0.21 mL/100 g/min; $P < 0.005$) (Figure 4). Little difference was seen between the 90° and 135° (used in Study 2) positions ($P > 0.05$).

Discussion:

In the comparison of the three methods of capsaicin application, the most consistent between-session MA and PPH areas were seen in the 36°C fixed temperature intradermal (36 ID) trials (Figure 2). This is consistent with the observations of Koltzenburg et al.[3] and Culp et al.[9] that MA areas are temperature sen-

sitive. Fixing the temperature in the intradermal model may lower variability and thus, decrease the required sample size in future studies using this model.

Topical application tended to produce MA and PPH of shorter duration and with smaller areas, despite the larger area of skin, and presumably larger number of nociceptors, activated by topical compared to intradermal capsaicin (Table 4). It is difficult to determine the exact amount the nociceptors were exposed to with the topical route as, though we could measure the spread of the solution, we could not measure the amount of capsaicin absorbed. Subjects who responded vigorously (areas of MA and PPH > 5 cm^2) to one form of administration tended to responded well to all forms of administration, and subjects who responded minimally to one form tended to respond minimally to all (Tables 1 and 2). The dosage actually received by the subjects with the topical administration may have varied. Using dye mixed with the topical capsaicin solution, Koltzenburg et al.[3] demonstrated different amounts of spread of the solution following placement of the patch. The actual area of skin contact with capsaicin was approximately one-third larger than the area of the patch. Given the spread associated with the topical method, it is possible that what is thought to be MA in the secondary zone in some subjects in our study may in actuality have been MA in the primary area, accounting for some of the variability.

This study and a previous study from our laboratory[7] report a large proportion of non-responders. Previous investigators did not report the presence of poor CAP-induced MA responders.[1–5,9] In our sample of 23 subjects, 48% had less than 5 cm^2 of MA in at least four of six (Study I) or three of four (Study II) sessions. The large proportion of non-responders may be partly explained by the definition of MA used and by the method of assessing MA. In these studies, subjects were instructed to indicate where they felt the brush to just begin to cause pain or discomfort. Other investigators instructed their subjects to indicate where a change in sensation is noted, a more liberal criterion.[3] The stimulus used to test for MA is also different from those used previously. The cotton swab on the thin metal strip as used by LaMotte et al.[2] and Koltzenburg et al.[3] causes more of a pressure sensation than the small brush used here, possibly activating A-delta and deep as well as superficial A-β fibers. We also observed sensitivity to pressure in our subjects as demonstrated by increased pain behavior (flinching, grimacing, vocalization) with the use of the pressure-evoking marking pen. All subjects indicated that the marking pen generated a much greater pain sensation than the brush in the same areas where brush MA was noted.

We also used a larger dose of intradermal capsaicin (250 μg) in Study I than investigators in previous studies. Some subjects without MA reported difficulty distinguishing different pain sensations, that is, MA and PPH, due to the severity of the ongoing pain. However, these same subjects did not later develop MA when ongoing pain had subsided. Interestingly, unlike previous investigators,[1] we did not see a correlation between MA or PPH areas and ongoing pain. Again, this may have been an effect of the different doses of capsaicin between the previous study and our study or due to the large number of non-responders in our study.

Of interest is the difference between Studies I and II in the between-session consistency observed with the forearm site. This may be a dose-related effect of capsaicin. In Study 1 using 250 μg, a between-session consistency of $r = 0.92$ was observed. In Study 2, with 100 μg, the between-session consistency decreased to $r = 0.49$. However, 100/μg intradermal capsaicin on the foot dorsum ($r = 0.84$) appears to give similar between-session consistency as 250 μg intradermal on the forearm.

Despite a significantly larger area of MA found on the foot dorsum compared to the volar forearm, four of twelve of the tested subjects had minimal MA at either site. Almost all of the subjects had pinprick hypalgesia. Upon further testing, some of these subjects reported areas of decreased pinprick sensation that extended outside the area of the receptor fields of the C-fibers stimulated by the capsaicin. This hypalgesia may have contributed to the subjects' lack of MA and PPH responses. Though a similar, non-dermatomal area of hypalgesia has been seen in patients with nerve injuries and surgical lesions,[12–14] it has never been reported previously in association with the CAP model. As suggested by Marchettini et al.[14] this

type of hypalgesia may result from a central disruption of pain processing.

Through use of the laser Doppler, we were able to demonstrate that decreased blood flow is present in the foot dorsum with the foot in the dependent position as compared to the reclining position. We hypothesize that this is due to increased sympathetic tone when the foot is in the dependent position. We report elsewhere[15] that sympathetic blockade with phentolamine infusion decreases the area of capsaicin-evoked MA (but not PPH or injection-evoked pain). This suggests that higher levels of sympathetic activity, as would be present with the foot in the dependent position, might promote the appearance of capsaicin-evoked MA. As the sympathetic nervous system has been suggested to play a role in pain,[16–20] the use of the foot dorsum may serve as a good location to evaluate the effects of the sympathetic nervous system on mechanical allodynia.

Intradermal CAP is a promising model for studies of MA and PPH. However, the variation in the size of MA and PPH areas among subjects can decrease the sensitivity of the model, affecting its utility as a model for studies of pain mechanisms and analgesic drug candidates. We have shown that controlling the skin temperature can improve the sensitivity of the model. Prescreening subjects for MA and PPH responses prior to entry into the study and excluding any subject with small areas of MA may further increase this sensitivity. We recommend a minimal MA area of 5 cm^2 for 15 min as a criterion for an adequate responder. Areas smaller than this may be difficult to distinguish from the primary pain areas. Injecting capsaicin before the experimental manipulation is made can also reduce variability. If minimal MA is produced, the experiment can be aborted and if adequate sensory changes result, one can normalize measurements that follow the intervention to the pre-intervention post-capsaicin baseline values.[7]

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